Antioxidants are not identical and their effects are not uniform over the population

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Comment on:

Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases.

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C.

Cochrane Database Syst Rev. 2008;(2):CD007176
http://dx.doi.org/10.1002/14651858.CD007176
http://www.ncbi.nlm.nih.gov/pubmed/18425980

This comment was published in **Cochrane Database for Systematic Reviews:**

Hemilä H.
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[Feedback Submitted 24 August 2009]
Cochrane Database Syst Rev
http://dx.doi.org/10.1002/14651858.CD007176 (see **Feedback** section)

Bjelakovic et al. replied to these comments and their replies are available at the above document and at the 2012 update of the Cochrane review:
http://dx.doi.org/10.1002/14651858.CD007176.pub2 (see **Feedback** section)
The replies are also available at:
http://www.mv.helsinki.fi/home/hemila/H28P.pdf

Not all of the replies are satisfactory, but this is not a place to analyze the replies by Bjelakovic et al. This document is the 2009 Feedback as it was submitted, except that the review group made minor changes such as replacing “Bjelakovic” with “Bjelakovic and coworkers” etc.

There are two fundamental problems with the review by Bjelakovic.
First, the authors are combining apples and oranges.
Second, the authors ignore the evidence indicating that vitamin E effect is not uniform over the population.

First, let us consider an analogy. If a researcher is interested in the effect of antibiotics on mortality caused by infections, and combines all antibiotics to a single broad category of "antibiotics", and pools all "antibiotic trials" together, people with basic background in clinical microbiology would consider such a project silly. Different antibiotics kill different bacteria, there is geographic and social group variation in the occurrence of pathogenic bacteria, the usage of antibiotics generates resistant strains, etc., etc. The biology of antibiotics is very complex. It is obvious that a single universal estimate for "antibiotic effect" is meaningless.

Antioxidants are also a heterogeneous group. Vitamin C is water soluble, vitamin E is fat soluble, selenium is an inorganic element, beta-carotene is not an essential nutrient, etc. Moreover, in model systems small-molecule antioxidants are oxidized at different speeds. For example, activated neutrophils in plasma first oxidize vitamin C, whereas the oxidation of urate starts only after vitamin C has been consumed, and the level of vitamin E remains virtually unchanged [1]. From the point of view of biology, there is no basis to consider that all antioxidants are similar enough to justify the pooling of all trials with compounds belonging to the broad category of "antioxidants." In a proper analysis, each antioxidant should be analyzed specifically.

Bjelakovic labels vitamin A as an antioxidant. Halliwell and Gutteridge write in the latest edition of their monograph, that *"vitamin A can do the same [scavenging some oxidants, as beta-carotene does] but no data exist supporting such a role in vivo"* [2 p. 177]. Of course, Halliwell and Gutteridge may be wrong, but given the depth of their familiarity with the antioxidant literature, Bjelakovic should give explicit reference(s) to reject that statement when labeling vitamin A as an antioxidant. Most biomolecules are oxidized by hydroxyl radical, but it is not reasonable to thereby label all of them as antioxidants. Halliwell and Gutteridge suggest that the definition of "antioxidant" should require that it delays, prevents or removes oxidative damage [2 p. 80-81]. Thus, a systematic review focusing on antioxidants should consider and define what is meant by an "antioxidant." Bjelakovic writes that *"there are pros and cons in the literature about vitamin A being antioxidant"* which in my opinion is not a scientific argument to justify labeling vitamin A as an antioxidant. Furthermore, Bjelakovic writes in Discussion that *"recent research revealed that vitamin A can cause oxidative damage to DNA"*. Thus, there is lack of logic when claiming that vitamin A has been shown to be a pro-oxidant, but include it in the review restricted to antioxidants on the basis of conflicting opinions.

Analyzing trials by the specific biochemical substance that is tested would make the review much more logical. If a review focuses on, say, vitamin A and mortality, there is no need to firmly decide whether vitamin A is an antioxidant or not. The problem of defining "antioxidant", and deciding whether some of them is pro-oxidant under certain condition is avoided if the substances are analyzed by the biochemical definitions. In such an approach, antioxidant and pro-oxidant concepts could be left to the Discussion section for giving plausible biological explanations for the observed effects.

An important goal in modern biomedicine is specificity. The strength of the randomized trial is that the difference between the trial groups can be specifically attributed to the intervention that is being tested. However, when the intervention varies from a single antioxidant to the combinations of diverse antioxidants, and includes 11 trials in which *"participants were supplemented with different mixtures of antioxidants as well as with vitamins and mineral without antioxidant properties"*, we lose specificity because of the apples and oranges problem.

In Table 6, Bjelakovic does calculate the specific effect of vitamin A (95% CI for the RR: 0.84 to 1.68; N=2406) and vitamin E (95% CI for the RR: 0.98 to 1.05; N=41341), but these specific

effect estimates are hidden from the reader. In the Discussion, Bjelakovic states that *"beta-carotene, vitamin A, and vitamin E given singly or combined with other antioxidant supplements significantly increase mortality"* which is false and misleads those readers who skip Table 6 and only look at the Discussion. The above confidence intervals show that vitamins A and E singly do no significantly increase mortality. In the Abstract, Bjelakovic does not give the specific estimates for vitamins A and E, but gives RR estimates based on studies with scores of other antioxidants including beta-carotene, which has been known to increase mortality since the publication of the ATBC and CARET trials. A reader of the Abstract cannot figure out that the given RR-values don't tell us anything about the specific effects of vitamins A and E. Thus, even the Abstract is misleading.

Second, when Bjelakovic first published the review in JAMA, I pointed out that the effect of vitamin E on respiratory infections was heterogeneous in the large scale ATBC Study [3,4]. Vitamin E had no overall effect on the incidence of the common cold or pneumonia, but the effects were significantly modified by age and smoking [5,6]. Although heterogeneity in the effect on respiratory infections does not directly imply that the effect on mortality must be heterogeneous, such a possibility should not be dismissed. If the effect of vitamin E is heterogeneous, then a single estimate for effect can be meaningless. However, Bjelakovic ignores this issue.

Motivated by our findings on respiratory infections, we analyzed the effect of vitamin E on the mortality of ATBC participants and found strong evidence that the effect of vitamin E on total mortality was also heterogeneous [7,8]. Vitamin E had no effect on those who had low dietary vitamin C intake; however, among those who had high dietary vitamin C intake, vitamin E increased mortality in young and decreased mortality in old participants. Close to half of the participants fell to those groups in which vitamin E effect was inconsistent with the average effect of the whole study population. When the average effect of a large trial is misleading for half of the study participants, it seems obvious that calculating and presenting a single universal "estimate for vitamin E effect" is an unsound approach.

Although heterogeneity in vitamin E effect on mortality does not directly imply that the effect of vitamin C and beta-carotene must be heterogeneous, such a possibility should not be ignored. In fact, we can even turn the argument around. Given the strong evidence that vitamin E effect is heterogeneous, why should we accept such a premise that the effects of other antioxidants are uniform over the population. If we assume that the effects of antioxidants are heterogeneous, further studies should try to identify and characterize the subpopulations where the antioxidants might be beneficial, rather than calculating a fictionally accurate average effect on all people. Lack of uniformity in vitamin C effect is suggested by the interaction between vitamin E supplementation and dietary vitamin C intake. Although dietary vitamin C has a high level of correlation with other substances in fruit and vegetables, the other substances did not explain the modification of vitamin E effect in the ATBC cohort [7].

Bjelakovic writes in Discussion that *"our analyses had little trial heterogeneity. This increases the trustworthiness of our findings."* I cannot see any justification for such an argument. If there is a strong premise that the effect of, say, vitamin E should be uniform over the population, in such a case observing little heterogeneity is consistent with our expectations. However, as noted above, there is no basis for such a premise in general, and in the case of vitamin E it was firmly refuted [7]. The level of heterogeneity is no measure of "trustworthiness."

Bjelakovic states in Discussion that *"adoption of the random-effects model in meta-analysis permits extension of inferences to a broader population of studies than the fixed-effect model does"* which is incorrect. If there is heterogeneity, we do not know to whom the calculated overall estimate applies, and this problem does not disappear by using the random-effects model. In the random-effects model the confidence interval is wider, but that does not help us to understand what are the characteristics that modify the effect: to whom there is effect and to whom not. When there is

evidence of heterogeneity, the main focus should be on trying to understand any sources of heterogeneity that are present [9].

Bjelakovic also concludes that the effect of “antioxidants” is uniform over the duration of supplementation: *“we found no significant effect of treatment duration on our results”* (Discussion). Our analysis of the individual-level data of the ATBC study refuted also this conclusion. In young participants who had high dietary vitamin C intake, vitamin E supplementation had no effect over the first 3.3 years, but thereafter increased mortality by 38% [7]. Adding the two different vitamin E effects significantly improved the Cox model (P=0.007). Correlation of treatment effect with the average duration of supplementation at the trial level is too crude a method to examine the time dependency of supplementation effects. In epidemiology, “ecological fallacy” means thinking that relationships observed for the averages for groups necessarily hold for individuals. Thus, Bjelakovic's conclusion that treatment duration has no effect on the effect of antioxidant supplementation is an example of the ecological fallacy.

Finally, Bjelakovic was ambitious when covering all antioxidant+vitamin A trials. Such wide coverage requires lots of work and easily leads to errors in the extraction of data, and to the lack of time to read the papers and learn the context of the trials. As a reflection of this problem, Bjelakovic wrote half-a-page erratum to their JAMA paper [10]. Nevertheless, in the 2008 version of the Cochrane review Bjelakovic still includes the Chandra 1992 trial [11] in their analysis, even though it had been shown to be fabricated several years earlier. The story should be familiar to everyone who follows the major journals [12-15]. In the reference list, under the citation of the Chandra 1992 report, Bjelakovic cites the Lancet letter [12]. Apparently, Bjelakovic lacked time to read the Lancet letter to see that there would have been good reasons to exclude the 1992 study from analysis.

Bailar criticized the meta-analysis approach in general and gave examples of severe errors in five influential meta-analyses [16]. In particular, Bailar criticized the “job-shop” approach: a group of researchers picks a topic, rushes to collect trials and pools their results, without making themselves familiar with the biology and other relevant context of the topic. Lack of considering the differences between antioxidants, labeling vitamin A as an antioxidant (while simultaneously claiming that vitamin A is a pro-oxidant), ignoring the evidence of heterogeneity in vitamin E effect, the large number of errors in the first version of the meta-analysis [10], the inclusion of the Chandra 1992 trial; all these indicate to me that there is a severe “job-shop” type of problem in Bjelakovic's review.

I do not disagree with Bjelakovic about the main conclusions. So far, there is no good evidence indicating that ordinary people would benefit from taking “antioxidant” supplements for the purpose of reducing mortality. This conclusion can be reached by reading the major trial reports separately, without calculating a fictional “pooled antioxidant effect.” In this respect, pooling of the results does not give us any additional understanding. Although the evidence of heterogeneity in the vitamin E effect on mortality is strong [7], I do not think that it justifies practical conclusions yet. Rather, the complexity encourages caution in drawing conclusions and patience in waiting for further research.

Bailar commented that *“meta-analysis can aid in filling in the second and third decimal places once the questions are clear … but it is a poor tool for developing new concepts, new hypotheses”* [16]. The Bjelakovic meta-analysis implies that there is no justification for further research on vitamin E and mortality because the particularly narrow confidence interval (0.98 to 1.05) firmly rejects any substantial benefits. In contrast, our analysis of the ATBC Study suggests a path that should be explored: does the combination of vitamins E and C improve the health of some subpopulations of elderly males. In this respect, my conclusions significantly diverge from those of Bjelakovic. Therefore the two problems discussed above are fundamentally important.

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